UNITED STATES DEPARTMENT OF AGRICULTURE
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A SIMPLE PHOTOMETRIC METHOD FOR DETERMINING THE PROTEIN CONTENT OF WHEAT FLOUR

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The Kjeldahl method, or one of its various modifications, is used extensively for the routine determination of protein in wheat and wheat flour. The method is reliable, fairly rapid, and is well adapted to routine work. Certain shortcomings of the Kjeldahl method, however, definitely limit its usefulness. In order to perform a large volume of work, a protein laboratory must be equipped with elaborate and very expensive equipment which must be permanently installed and is, therefore, nonportable. Large quantities of strong acid and alkali must be used, and noxious fumes and excessive heat are characteristic of most protein laboratories.

The Agricultural Marketing Service is interested in the development of some simplified procedure by which protein determinations may be made conveniently in small laboratories without the installation of cumbersome equipment. Such a method may be of considerable value in certain types of grain-inspection work.

The method herein described has been developed by utilizing certain physico-chemical properties of the gluten proteins, and is not based on any previously established procedure. It should be mentioned, however, that several colorimetric and turbidimetric methods for determining the protein content of various body fluids have been developed in recent years and are being used to advantage in clinical laboratories for the routine analysis of blood serum, urine, and cerebro-spinal fluid. Since the photometer is used with success in some of these clinical methods, it was believed that a photometric method might be developed for the determination of protein in cereal products.

The proteins of wheat flour are readily peptized by very dilute alkali. When an alkaline solution containing the peptized flour proteins is neutralized, the gluten proteins are precipitated. If, however, the protein sol is sufficiently dilute, and its hydrogen-ion concentration after neutralization is controlled by a suitable buffer, the precipitated gluten proteins will not separate from the solution but will remain dispersed in the form of a highly stable colloidal suspension. The degree of turbidity of such a suspension is a measure of the gluten protein content of the flour.

Experimental Work

To determine the optimum hydrogen-ion concentration and the minimum time required for the production of the greatest turbidity in a neutralized alkaline extract of wheat flour, 5 ml. portions of a filtered

0.05 N. potassium hydroxide extract of a flour were added to 25 ml. portions of a series of buffer solutions. The buffers covered a range in pH values from 6.0 to 8.7, and were prepared by mixing M/5 solutions of $Na_2H\ PO_4$ and $KH_2\ PO_4$ in various proportions. The relative degrees of turbidity of the suspensions produced were determined by measuring the light transmission through a definite depth of suspension by means of a photoelectric photometer, using a light filter with a maximum light transmission at a wave length of 530 millimicrons. The values obtained are given in table 1.

It is evident that under the conditions used the maximum turbidity (minimum light transmission) is attained by using a buffer of pH 7.8, which results in a suspension having a pH of approximately 8.2. It is also evident that the maximum turbidity occurs in about 60 minutes after the addition of the buffer to the extract. This time, however, is not critical, since the change in turbidity between 45 and 90 minutes is very small. It was further shown that the temperature of the suspensions had very little effect on the final degree of turbidity, at least within the ordinary range of laboratory temperature. For very exact work, however, it is possible that the temperature should be controlled or that a temperature correction factor should be used.

The following method, based on the foregoing experiments, was used to determine the relationship between the turbidity produced and the protein content of the flour.

(1) To exactly 0.5 gm. of flour in a 200-ml. Erlenmeyer flask, add exactly 5 ml. of 95 percent ethyl alcohol (to prevent the flour from coalescing) and 100 ml. of 0.05 N. KOH.

(2) Shake mixture intermittently for 15 minutes and then centrifuge for 10 minutes at approximately 1600 R.P.M.

(3) To exactly 5 ml. of the centrifugate in a photometer test tube (one of the selected tubes for use in lieu of an absorption cell), add exactly 25 ml. of a buffer solution made by mixing 6 parts by volume of M/5 $KH_2\ PO_4$ with 94 parts by volume of M/5 $Na_2H\ PO_4$. This buffer solution should have a pH of 7.8. Mix the contents of the test tube by inversion and allow to stand for 1 hour.

(4) Determine the transmission of light through the solution in the test tube with a photoelectric photometer, using a light filter having a maximum transmission at a wave length of 530 millimicrons. (Other wave lengths will give different but equally as satisfactory results.)

Table 1.-- Transmission of light at 530 m.mu. through suspensions prepared by adding 5 ml. portions of a filtered 0.05 N. KOH extract of a wheat flour to 25 ml. portions of M/5 phosphate buffers. Suspensions contained in matched 7 by 7/8-inch test tubes

Suspension number	Buffer formula - M/5 Na_2HPO_4	Buffer formula - M/5 KH_2PO_4	H-ion concentration - Buffer	H-ion concentration - Suspension	Light transmission after - 15 min.	45 min.	60 min.	90 min.	24 hr.
	Volume	Volume	pH	pH	Percent	Percent	Percent	Percent	Percent
1	100	0	8.72	9.63	62.3	60.4	59.9	59.6	63.4
2	99	1	8.39	9.47	57.7	55.6	55.3	55.2	56.7
3	97.5	2.5	8.10	9.10	55.1	53.5	53.2	53.2	53.7
4	95	5	7.86	8.30	52.9	52.0	51.7	51.7	52.8
5	94	6	7.80	8.16	52.3	51.5	51.2	51.3	51.9
6	93	7	7.71	8.02	52.8	51.9	51.6	51.8	52.5
7	92	8	7.63	7.83	53.2	52.0	52.0	52.2	52.8
8	91	9	7.60	7.76	53.5	52.5	52.2	52.3	53.2
9	90	10	7.51	7.62	54.5	53.1	52.7	52.7	53.2
10	80	20	7.23	7.31	55.5	54.3	53.9	53.9	55.3
11	70	30	6.98	7.08	56.7	55.1	55.1	55.1	56.1
12	60	40	6.79	6.88	57.5	56.6	56.5	56.3	57.7
13	50	50	6.61	6.72	60.1	59.4	59.0	58.9	60.6
14	40	60	6.43	6.50	62.7	62.2	61.9	61.8	65.6
15	30	70	6.26	6.36	66.9	66.6	66.4	66.3	86.4 1/
16	20	80	6.03	6.18	68.9	68.5	68.2	68.2	92.3 1/

1/ Precipitate settled out.

Thirty-four samples of flour representing all classes of domestic wheat except durum were analyzed by this method. These flours ranged in protein content from 7.30 percent to 16.26 percent, and in ash content from 0.38 percent to 0.61 percent. The results are given in table II and are shown graphically in comparison with the protein content values as determined by the conventional Kjeldahl method in figure 1.

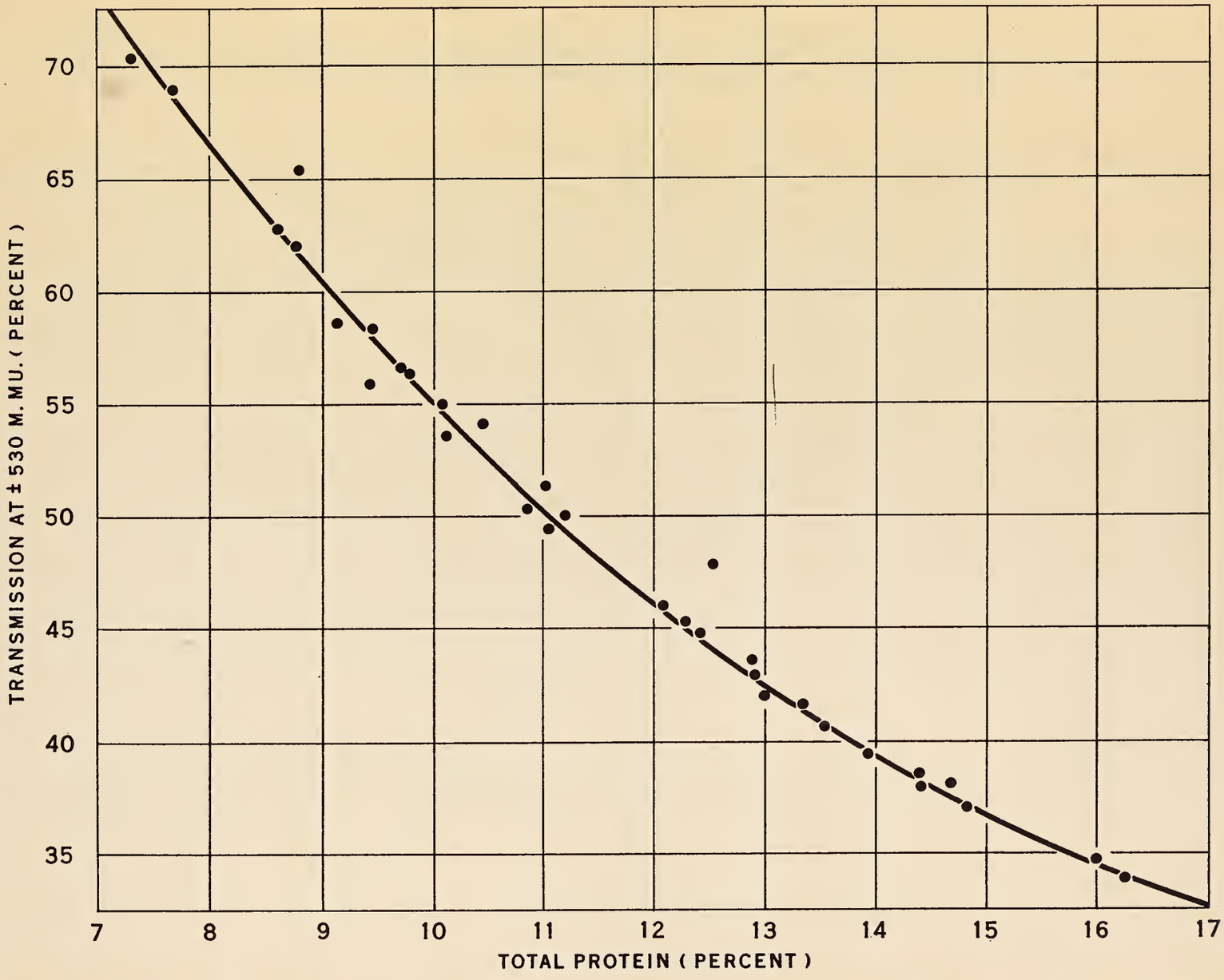
From the curve in figure 1 it is evident that the protein content can be predicted rather accurately from the light transmission values. Only 3 of the 34 flour samples show deviations from this relationship of more than 0.3 percent in terms of protein content, the mean deviation being 0.15 percent protein.

Since the turbidity produced in these suspensions is caused by the gluten proteins only, while the albumin and globulin remain completely dispersed, any variation in the normal ratio of gluten to nongluten protein will cause a deviation in the relationship between total protein content and light absorption. Theoretically, therefore, the light absorption values should be an even better index of gluten protein content than of total protein content.

To test this theory the protein peptizable in 5 percent potassium sulfate solution was determined on each of the 34 samples of flour under investigation, and by difference the protein not peptizable by the same salt solution was determined. This latter protein fraction may be considered an approximate measure of the gluten protein content, since the gluten proteins are not appreciably dispersed by 5 percent potassium sulfate. These values also are given in table II and are shown graphically in figure 2.

A comparison of figures 1 and 2 shows a better relationship between light transmission and protein not peptizable with 5 percent potassium sulfate than between light transmission and total protein. The mean deviation was reduced to 0.13 percent protein and only 1 of the 34 samples deviated by more than 0.3 percent protein from the general relationship as shown by the curve in figure 2. It, therefore, appears that the photoelectric method is a slightly more exact measure of gluten protein content than of total protein content of wheat flour, and should therefore be a slightly better index of baking quality than the total protein content as determined by the Kjeldahl method.

A protein determination by the photometric method as described may be made in about the same length of time as by the Kjeldahl method, and may be made in considerably less time with but little sacrifice in accuracy by allowing only 15 minutes for the development of turbidity. For routine work the photometric method should prove considerably more rapid and less fatiguing to the analyst than the Kjeldahl method.



U. S. DEPARTMENT OF AGRICULTURE

NEG. 242 AGRICULTURAL MARKETING SERVICE

Figure 1. - Relationship between total protein content and light transmission of protein suspension for 34 samples of wheat flour.

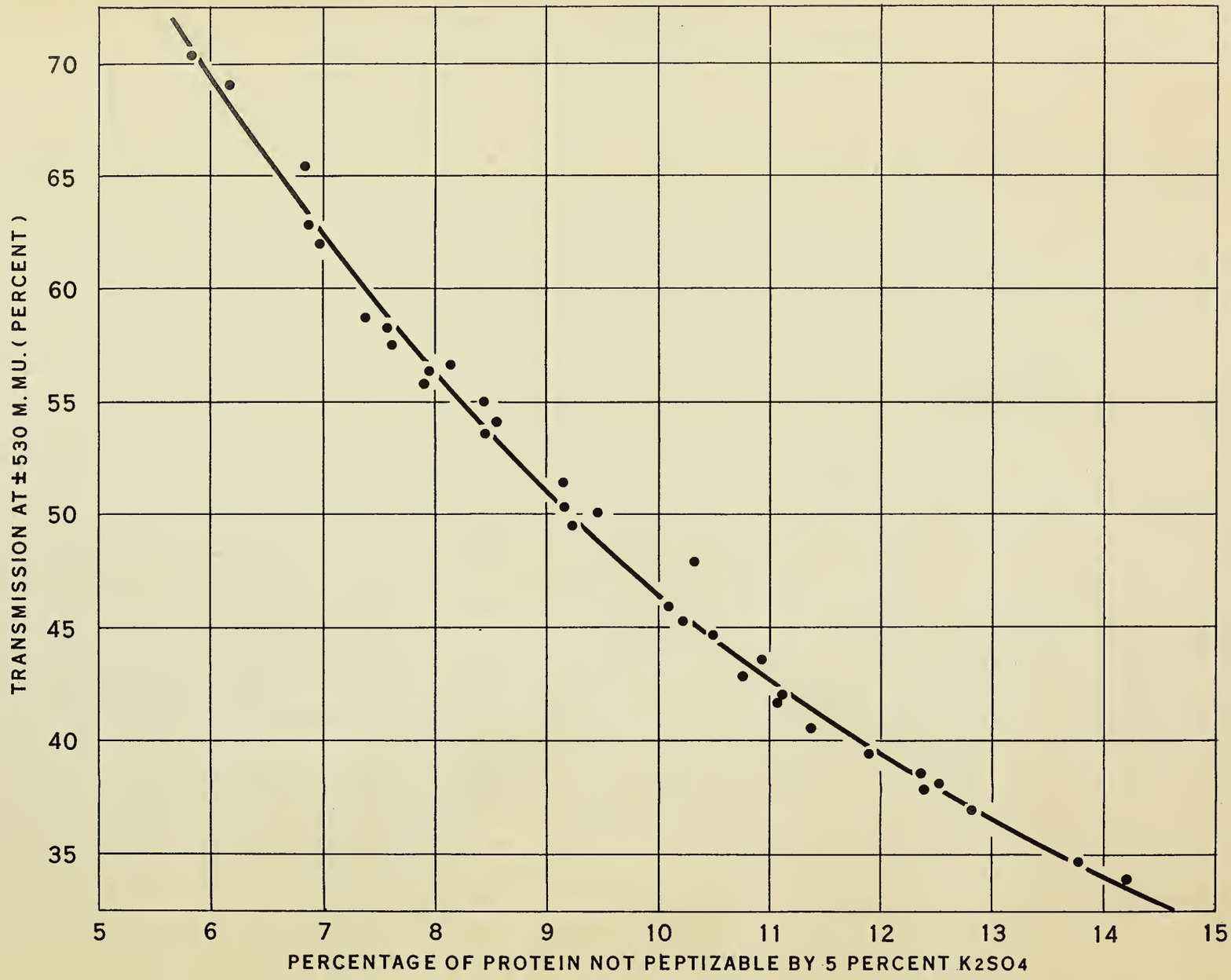


Figure 2. - Relationship between content of protein not peptizable by 5 percent K_2SO_4 ("gluten" protein) and light transmission of protein suspension for 34 samples of wheat flour.

Table II.- Showing the relationship between protein content and light transmission values on a series of 34 flour samples

Sample No.	Class and variety of wheat from which flour was milled	Protein content (as is basis)		Light transmission at +530 m mu. (In 7 by 7/8-inch test tubes)
		Total	Not peptized by 5 percent K_2SO_4	
		Percent	Percent	Percent
1	White, (Other than Federation or Baart)	7.30	5.85	70.4
2	White Club	7.66	6.18	69.0
28	White	8.61	6.89	62.8
29	White	8.77	6.98	62.1
3	White Federation	8.80	6.86	65.4
32	Soft Red Winter, Purkoff	9.13	7.39	58.7
34	Soft Red Winter, Nittany	9.31	7.63	57.6
33	Soft Red Winter, Red Rock	9.44	7.59	58.3
4	Hard Red Winter	9.44	7.91	55.9
5	White (Other than Baart)	9.70	8.16	56.7
26	White	9.79	7.96	56.4
6	White, Baart	10.09	8.46	55.0
19	Hard Red Spring	10.13	8.46	53.7
10	(Unknown)	10.47	8.57	54.2
7	Hard Red Winter	10.86	9.19	50.4
24	Hard Red Spring	11.01	9.18	51.4
30	Hard Red Winter, Minnturki	11.05	9.27	49.5
23	Hard Red Spring	11.18	9.51	50.1
31	Hard Red Winter, "2614"	12.08	10.10	46.1
14	Hard Red Spring, Marquis	12.29	10.25	45.4
16	Hard Red Spring	12.39	10.50	44.8
8	White, Baart	12.52	10.37	47.9
20	Hard Red Spring	12.88	10.96	43.7
12	Hard Red Spring, Sturgeon	12.90	10.77	42.9
17	Hard Red Spring	13.00	11.14	42.1
11	Hard Red Spring, Progress	13.34	11.09	41.7
13	Hard Red Spring, Thatcher	13.55	11.38	40.7
18	Hard Red Spring	13.94	11.92	39.4
9	Hard Red Spring	14.39	12.36	38.6
25	Hard Red Spring	14.41	12.42	37.9
27	White	14.68	12.53	38.2
21	Hard Red Spring	14.82	12.83	37.1
22	Hard Red Spring	16.00	13.78	34.7
15	Hard Red Spring	16.26	14.22	33.9

No table for converting photometric readings into protein percentages is presented herewith, since there is some question as to the uniformity of different makes of photometers, and since the results herein reported were obtained by the use of selected test tubes in the photometer instead of precision absorption cells. The use of such test tubes when well matched is highly desirable for routine work, and the results obtained are of the same order of accuracy as when precision cells are used. However, a conversion table based on such a series of test tubes cannot be expected to apply to any other series of matched tubes. For the present, therefore, it is recommended that in the application of this photometric method each instrument be calibrated against the standard Kjeldahl procedure for a series of flour samples.

Conclusions

A photometric method for determining the protein content of wheat flour has been developed. The results are in good agreement with those obtained by the standard Kjeldahl procedure and appear to be a somewhat better measure of gluten protein than of total protein content.

The principal advantages of the photometric method for routine work should be the ease and rapidity with which a large volume of work can be handled with a minimum amount of equipment, and without the unpleasant features usually associated with a protein laboratory.

Efforts will be made to adapt the procedure to the determination of protein in wheat as well as flour. The success of this effort would greatly enhance the practical value of the method.

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